IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Clarence N. Ahlem, et al.
Application No.	: 10/602,330
Filed	: June 23, 2003
Docket No.	: 202.2D2
Title	: Pharmaceutical Compositions and Treatment Methods
Examiner	: Barbara P. Badio
Group Art Unit	: 1617
Customer No.	: 26551
Confirmation No.	: 9052

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Christopher L. Reading, declare as follows:

1. I am a co-inventor of the above-referenced patent application and an employee of the assignee of this patent application. I have been engaged in the evaluation and development of therapeutic agents and treatment methods for over 25 years, which includes 8 years of experience with preclinical research and clinical development of steroid compounds. A summary of my resume is attached hereto as Attachment 1 and included as a part of this declaration. The following statements are based on the documents identified below, my knowledge of the experiments and results that are discussed below and/or my professional training and experience.

2. I have read U.S. patent No. 5,461,042 (the '042 patent). Based on that reading, I do not believe that it either teaches or suggests the use of any of the presently claimed methods, which specify (a) daily dosages that are above the

patent's explicit teaching and/or (b) dosing regimens that the '042 patent neither expressly nor inherently suggests. The amended claims recite the use of androst-5-ene-3β,17β-diol ("AED") to treat certain innate immune suppression conditions in humans or non-human primates (usually rhesus monkeys). The '042 patent contains no data showing any biological effect of AED in any human or non-human primate. The *in vivo* data in the '042 patent was obtained exclusively from mice. The '042 patent is silent on dosages and dosing regimens that might be used specifically for humans or non-human primates. As discussed in paragraph 3 below, the '042 patent describes dosages for "larger adult mammals". This does not specify or suggest any dose regimen or daily dosage for humans or non-human primates.

3. The '042 patent at column 17, lines 27-31 and column 17, lines 37-47 contains the following two passages:

> A preferred method of administration is by subcutaneous injection as a depot. The method is particularly appropriate for administration of the active agents to mammals, since subcutaneous injection is easily performed and the effect is relatively long lasting.

> The dosages used will depend on the size and condition of the host. Test data indicated in this application was obtained in small animals. In larger adult mammals daily dosage of 0.2 to 30 mg/da. of AED is a preferred dosage. For AET the preferred dosage is usually in the range of 0.001 to 20 mg/da, with 0.001 to 1 mg/da. being the more preferred dosage. However, the dosage will vary depending on the route of administration. Subcutaneous, inhalation and intrathecal administration are methods that would require lower dosages of the active agents.

Based on my experience in drug development and the plain meaning of the quoted language at column 17, lines 27-31 in the context of the rest of the patent, I believe that the '042 patent discloses a preferred dose regimen (or treatment protocol) as a single subcutaneous dose. It also expressly states that AED dosages of 0.2 to 30 mg/day are preferred for treating larger adult mammals, with

lower but unspecified dosages being sufficient for subcutaneous, inhalation or intrathecal administration. The daily AED dosages for "larger adult mammals" were specified as 0.2-30 mg/day as the passage at column 17, lines 38-47 states. Neither passage expressly or inherently says that one should increase the number of doses or the daily dosage for a human or for a non-human primate. Example 9 at column 14, lines 14-20 is consistent with this by describing a capsule containing 15 mg of AED that would be administered once or twice per day. This teaches a daily oral dosage of 15 mg/day or 30 mg/day, which is within the dosages specified at column 17, lines 37-47. To infer that the '042 patent at column 17, lines 37-47 suggests dosages that were higher than the preferred dosages is to read the first sentence at lines 36-37 at column 17 and ignore the rest of the passage at lines 37-40. In view of these considerations, I do not believe that the '042 patent expressly or inherently suggested that one of ordinary skill in the art should use either the claimed daily dosages or the claimed treatment regimens that the amended claims recite.

4. Based on my experience with drug development, I believe that the dosage of 0.2 to 30 mg/day for AED in larger adult mammals means that this dosage range is for oral administration. I believe this because all three of the named routes of administration that would require lower dosages, i.e., subcutaneous, inhalation or intrathecal, are parenteral or topical routes of administration. This is consistent with example 9 of the '042 patent at column 14, lines 15-26, which discloses capsules for oral administration that contain 15 mg of AED, which the '042 patent states is to be administered either once per day or twice per day. Collectively, when I read these portions of the '042 patent in view of the other examples and disclosure in the '042 patent, I see no express or implied suggestion to select either the dose ranges or dose regimens that are now claimed. The '042 patent does not suggest that one would want to increase a dose that is administered by a parenteral route such as by intramuscular or subcutaneous injection, when the patent expressly states that dosages, at least for subcutaneous administration, would "require lower dosages of the active

agents." The daily dosages the claims recite are higher than what the '042 patent teaches. Similarly, one of ordinary skill in the art would find no reason to arrive at the dose regimens that claims 106-118 recite, since the single dose protocols the '042 patent describes were adequate to obtain the results the '042 patent discloses. This is reinforced by the statement in the '042 patent at column 17, lines 28-32 which states that depot administration by subcutaneous injection is preferred since the effect "is relatively long lasting." This statement is consistent with the statement in the '042 patent at column 14, lines 3-4, which notes that AED is a lipophilic compound.

5. The '042 patent does not expressly or inherently disclose specific dosages, e.g., a 100 mg or 200 mg daily dose, that some of the claims recite. Nor does it expressly or inherently disclose any specific continuous daily dosing regimen with any number of specified days of dosing. The dosages and single dose treatments the '042 patent teaches for treatments with AED are insufficient to see the effects that are seen with higher daily doses in humans or the non-human primates as described below.

6. Two clinical trials were conducted to the activity of AED in humans. These phase I studies assessed the safety of AED in healthy adult volunteers and examined immune system effects *in vivo*. The compound was administered by intramuscular injection according to the protocols included with this declaration as Attachment 2. In the first trial, a single dose of 50 mg, 100 mg, 200 mg or 400 mg was administered. In the second trial AED was administered for 5 consecutive days using daily doses of 50 mg, 100 mg, 200 mg or 400 mg. The patients in the 5 dose trial weighed from about 50 kg (about 110 lb) to about 100 kg (about 220 lb). With respect to toxicities or adverse events, administration of AED was primarily accompanied by mild to moderate side effects associated with injection site irritation. There were no significant changes in blood parameters such as neutrophils after administration of a single 50 mg, 100 mg, 200 mg or 400 mg dose of AED. Analysis of various blood parameters from the second trial

revealed unexpected results, specifically increases in both neutrophils and platelets at the 200 mg and 400 mg dose levels. Administration of doses of AED that were higher than the 0.2 - 30 mg/day doses the '042 patent discloses for use in larger adult mammals were needed to obtain the neutrophil and platelet responses that were observed. The '042 patent also explicitly states that lower dosages would be needed for subcutaneous, inhalation or intrathecal administration of the compounds the patent describes.

7. Administration of AED at 200 mg/day or 400 mg/day generated a dose-related increase in neutrophils in circulation at Study Days 2-5. Patients treated with AED at 50 mg/day or 100 mg/day for 5 days showed neutrophil count increases, but the increases did not reach statistical significance compared to placebo controls. Peak neutrophil increases at Study Day 5 were observed in both the 200 mg and 400 mg dose levels. The table below shows the dose response related absolute neutrophil count on Study Days 2-5,14, 28 and 56. Neutrophil counts began to increase on day 2. By peak days 4-5 the absolute neutrophil count had increased by 85% in the 200 mg dose group and by 140% in the 400 mg dose group. By Study Day 14 neutrophils in the peripheral blood had returned to baseline. Increased neutrophil counts at days 2-5 are consistent with an increased release of cells from bone marrow to the circulation because neutrophil precursors mature and leave the marrow over this time period.

Absolute neutrophil count response

Study Day	Slope	p-slope	p-JT
Day 2	0.49	< 0.0001	< 0.0001
Day 3	0.66	< 0.0001	< 0.0001
Day 4	0.97	< 0.0001	< 0.0001
Day 5	0.74	< 0.0001	< 0.0001
Day 14	0.13	0.16	0.13
Day 28	0.11	0.21	0.24
Day 56	0.03	0.81	0.58

Slope: Regression coefficient for dose (cells/nL)/mg.
p-slope: significance of the slope in a linear regression model.
p-JT: significance of the exact nonparametric Jonckheere-Terpstra test for the presence of any upward or downward trend.

8. The neutrophil results were unexpected in part because AED elicited a neutrophil response in healthy adults with no overt immune suppression or neutropenia condition. Immune homeostasis mechanisms operate to maintain relatively constant levels of most immune components such as neutrophils absent a disorder or disease condition that perturbs the equilibrium (J.E. Layton et al., *Blood*, 74(4):1303-1307, 1989; J.F. Seymour et al., *Blood*, 90(8):3037-3049, 1997, newly cited). This data was evidence that AED could increase neutrophils in the face of opposing mechanisms to keep neutrophil levels relatively constant. This data is evidence that AED can be used to treat otherwise healthy humans that may be prone to develop innate immune suppression conditions, e.g., in elderly persons (e.g., T.P. Plackett et al., *J. Leukocyte Biol.*, 76:291-299, 2004, newly cited).

9. In addition to the neutrophil response described above, administration of AED induced a significant dose-related platelet increase with peak platelet activity observed on Study Day 14 in the 200 mg and 400 mg dose groups. The table below shows the dose response related absolute platelet responses. At peak (Day 14) platelets had increased by 30%. By Day 28 platelet counts had stabilized but were still above baseline. The timing of peak stimulation at Day 14 of platelets observed in this study suggested that AED acted by stimulating megakaryocytic progenitor cells, which take approximately 2 weeks to mature in the bone marrow. The table below shows the platelet response with a statistically significant increase at day 14.

Dose response in platelets (absolute)

Study Day	Slope	p-slope	p-JT
Day 2	-1.85	0.26	0.44
Day 3	1.09	0.61	0.58
Day 4	-1.13	0.63	0.46
Day 5	0.52	0.80	0.63
Day 14	19.56	0.0003	0.0001
Day 28	-1.74	0.46	0.55
Day 56	3.60	0.20	0.03

Slope: Regression coefficient for dose (cells/nL)/mg.
p-slope: significance of the slope in a linear regression model.
p-JT: significance of the exact nonparametric Jonckheere-Terpstra test for the presence of any upward or downward trend.

The Increases in platelets correlates with increased innate immune response capacity. Platelets directly mediate antibacterial responses (J.G. Hirsch, *J. Exp. Med.*, 112:15-22, 1960; A.L. Copley et al., *Thromb. Res.*, 8:251-262, 1976, newly cited). Platelets also prevent bleeding, which is associated with decreased bacterial sepsis (Stickney et al., 2007, in press; A.L. Copley et al., above). AED was thus capable of eliciting increases in innate immune responses by increasing both neutrophils and platelets in humans.

10. The activity of AED in non-human primates exposed to a sublethal dose of whole body radiation was examined in a series of studies. The protocols are described at Attachment 3. The neutrophil response associated with AED treatment was observed in these studies as a delay in the onset of neutropenia, a shortened duration of neutropenia and/or a reduced severity of neutropenia as compared to vehicle controls. For most of the studies, groups of animals were exposed to a target radiation dose of 400 cGy or 440 cGy (range 380 cGy-500 cGy) of whole body γ-radiation from ^{60}Co radiation sources. In one study, a linear accelerator (6 MV X-rays) was used as the radiation source. Vehicle alone or vehicle containing AED was administered by subcutaneous or intramuscular injection of aqueous suspensions. The dosing protocols, daily dosages, formulations and data from some of these studies are summarized at D.R. Stickney et al., *Int. Immunopharmacol.*, 6:1707-1713, 2006, newly cited. Results from these studies showed that AED dosed for 5 daily doses by intramuscular

injection of 7.5 or 15 mg/kg of an AED suspension formulation led to increased neutrophils and platelets at various times. Dosing with a suspension formulation that had an average AED particle size of D (v, 0.9) 1.1 μm at 15 mg/kg once per week for 4 weeks gave similar results to 5 consecutive daily 15 mg/kg doses particles at D (v, 0.9) 5.5 μm. The D (v, 0.9) value is the volume-weighted diameter at which 90% of the particles are at the specified size or smaller, e.g., a D (v, 0.9 1.1 μm) means that 90% of the AED particles were 1.1 μm in maximum length or smaller.

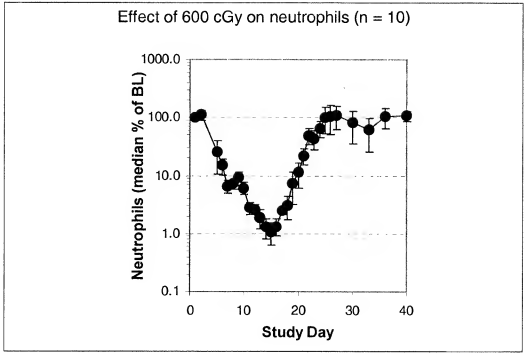
11. Other sublethal irradiation studies using ^{60}Co γ-radiation not described in Stickney et al., showed that (1) dosing for more than 5 sequential daily doses did not provide significant added benefit when compared to dosing with 15 mg/kg for 5 consecutive days and (2) dose levels of 5 mg/kg/day generally provided lower levels of benefit for neutropenia compared to dosing for 5 consecutive days at 10 mg/kg/day or 15 mg/kg/day. In one study, a group of animals was dosed once with 42.5 mg/kg and this single dose ameliorated neutropenia compared to vehicle controls as seen by a reduced neutrophil nadir and an earlier recovery from neutropenia. In one sublethal irradiation study, administration of AED reduced the group mean percentage of observations of very severe neutropenia at day 3 through day 36 from 41% (vehicle control group) to 17% (2.5 mg/kg for 10 daily doses), 20% (7.5 mg/kg for 10 daily doses) and 7% (42.5 mg/kg for 8 daily doses). Amelioration of neutropenia in individual animals was typically observed as a reduced neutrophil nadir, a reduced duration of neutropenia and, in some cases complete prevention of neutropenia. The reduced duration of neutropenia was seen as a delayed onset of neutropenia and/or an earlier recovery from neutropenia.

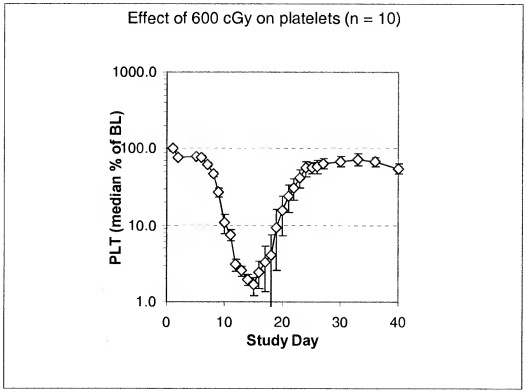
12. In addition to the sublethal irradiation studies discussed above, lethal irradiation studies were conducted with non-human primates exposed to 600 cGy, 620 cGy (1 study) or 634 cGy (1 study; 10 vehicle control animals and 10 animals treated with 15 mg/kg/day of AED for 5 consecutive days). The protocols

for the lethal irradiation studies are described at Attachment 3. Before the studies were conducted, the available data comprised studies on rhesus macaques using varying doses of total body irradiation with and without clinical support. Based on available data, a 600 cGy radiation dose was expected to be lethal for about 25-50% of irradiated animals without clinical support such as blood transfusions, platelet transfusions, electrolyte transfusions, antibiotics for infections or assisted feeding. Lethality in the range of 25-50% was expected from natural biological variability in individuals in the absence of clinical support. The 634 cGy study was used to evaluate the biological activity of AED after an high radiation dose with lethality expected at or above 50%. These studies allowed (1) assessment of the effect of AED on survival and the severity and duration of innate immune suppression (neutropenia and thrombocytopenia) in the face of high radiation exposure conditions without clinical support and (2) assessment of biological parameters associated with animals experiencing irreversible morbidity. The latter information was used to identify humane euthanasia criteria for animals *in extremis*. This was information used to meet regulations requiring study protocols with procedures that prevent unnecessary pain and distress.

13. Results from four 600cGy studies are summarized by D.R. Stickney et al., *Intl. Immunopharmacol.*, 7(--):---, 2007 (uncorrected proof), in press; published electronically Jan. 12, 2007, newly cited. In these studies, AED in suspension formulations were administered after irradiation once per day for 5 consecutive days at 5, 10 or 15 mg/kg/day or once on the day of radiation at 37.5 mg/kg. The 37.5 mg/kg dose data is not described in Stickney et al., 2007. Results from four animal treatment groups were combined to obtain sufficient numbers of animals to permit statistical analysis of the data. The analysis showed that treatment of irradiated animals with AED ameliorated neutropenia by significantly reducing the duration of neutropenia compared to vehicle controls ($p < 0.01$). Also as described in Stickney et al., 2007, AED treatment increased the survival rate to 87.5% in AED treated animals compared to a survival rate of

67.5% for vehicle control animals ($p < 0.032$), a 20 percentage point increase in the survival rate. Drug treatment also significantly decreased duration of thrombocytopenia ($p < 0.01$), which was significantly correlated with increased survival in the 600 cGy studies. Although the 5 mg/kg/day drug dose did not significantly increase survival (5% increase), this dose did result in 10-15% reductions in the cumulative mean days of very severe neutropenia ($p=0.04$) and acutely life-threatening neutropenia ($p=0.37$). A minimum effective dose for treating neutropenia of 5 mg/kg/day for 5 consecutive days was identified in the 600 cGy studies based on the neutrophil response. The single 37.5 mg/kg AED dose resulted in a 25 percentage point increase in survival when compared to the vehicle control group, which appeared to be associated with a protective effect on neutrophils (neutrophil nadirs were 0/μL in vehicle controls and 9/μL for AED treated). The single 37.5 mg/kg dose decreased the cumulative incidence of mean days of very severe neutropenia from 12.0 to 8.0 days and acutely life-threatening neutropenia decreased from 8.0 to 5.5 days. In the 634 cGy study, AED treatment qualitatively decreased the cumulative incidence of mean days of very severe neutropenia by 2.3 days ($p = 0.103$) and the cumulative incidence of mean days of acutely life-threatening neutropenia by 1.5 days ($p = 0.053$). The survival rate for AED treated animals was 40% compared to 20% for the vehicle control group in the 634 cGy study. The course of neutropenia and thrombocytopenia in untreated vehicle control animals ($n = 10$) after exposure to 600 cGy of radiation is shown below for the pilot study with no AED treatment arm.





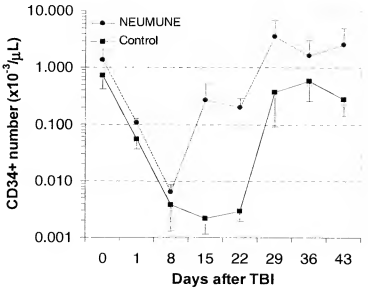
14. To my knowledge, the results described in paragraph 13 represent the first demonstration that a single drug could treat neutrophil deficiency and

increase survival in lethally irradiated primates without clinical support. Obtaining evidence of efficacy after lethal irradiation for other therapies had always relied on providing clinical support in the form of antibiotic treatments and/or blood or platelet transfusions to affect cytopenia or survival after lethal irradiation, e.g., T.J. MacVittie et al., *Blood,* 87(10):4129-4136, 1996; of record. Providing clinical support to irradiated animals complicates assessment of efficacy since such support alone can increase survival, e.g., MacVittie et al., above. Results in the 600 cGy protocol studies in non-human primates showing a significantly reduced duration of neutropenia and a significant increase in survival based on a monotherapy without clinical support were both unprecedented and unexpected.

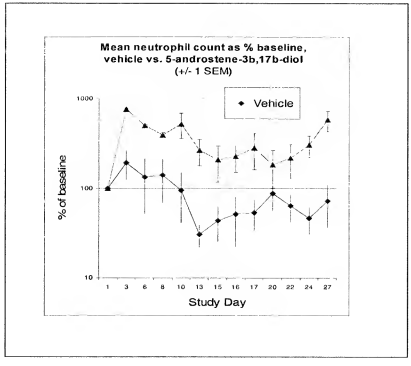
15. A number of different suspension formulations, AED particle sizes and dosing regimens were examined for activity *in vivo*. One formulation that was used in several non-human primate irradiation studies did not elicit a significant neutrophil or platelet increase compared to vehicle controls. In this particular formulation, analysis showed that one excipient (polysorbate 80) was found to contain peroxides, which generated a number of breakdown products over time. The breakdown products in the formulation were associated with the lack of a neutrophil or platelet response after irradiation. To limit formation of the breakdown products, AED suspension formulations were subsequently made with purified polysorbate 80 that lacked an appreciable amount of peroxide. The suspension AED formulations that were examined for particle size had a range of particle sizes from D (v, 0.9) 1.1 μm to D (v, 0.9) 38.3 μm. Particles in one preparation had input particles of D (v,0.9) 84 μm, but particle size in the final formulation was not determined. For this range of particle sizes, AED suspensions administered at 15 mg/kg/day for 5 days by subcutaneous or intramuscular administration to non-human primates after irradiation effectively ameliorated neutropenia. The apparent activity associated with various AED particle sizes was generally similar although smaller particle sizes qualitatively tended to give a better neutrophil response. Results from protocols with a 400 cGy dose level and AED particle sizes in this size range lead to selection of a

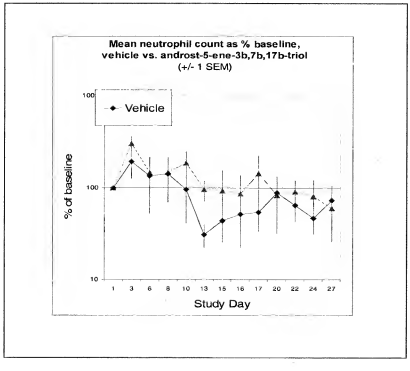
preferred administration regimen of dosing once daily on days 1-5 with a dose of 15 mg/kg/day. Comparison of a single 75 mg/kg AED dose after 400 cGy irradiation with the same total dose mass of 5 consecutive 15 mg/kg/day AED doses gave similar neutrophil responses and ameliorated neutropenia compared to vehicle controls, which indicated that a sufficiently high single dose of AED would ameliorate neutropenia in a manner similar to dosing for 5 consecutive days with a lower daily dose.

16. A 600 cGy irradiation study with clinical support was conducted to observe recovery of bone marrow in AED treated animals. Details of this protocol are shown at Attachment 4. In this study, AED was administered at 15 mg/kg/day by intramuscular injection (n=4) or placebo vehicle control (n=4) beginning at 2 hours after irradiation and daily for four more days. The protocol in this study differed from the 600 cGy protocols described above by providing standard clinical care, antibiotics, whole blood transfusions and/or electrolytes when the clinical status of the animals reached thresholds that triggered initiation of support. Administration of 15 mg/kg/day of AED had an early stimulating and/or protecting effect on the $CD34^+$ stem cells in bone marrow, leading to accelerated recovery of red blood cells, white blood cells and platelets. Clinically, this was reflected by a decreased need for blood transfusions and antibiotic treatments. Data analysis showed increases in neutrophils ($p = 0.0286$, Wilcoxon Mann-Whitney test) and $CD11b^+$ neutrophil precursor cells. Analysis of $CD34^+$ stem cells by difference in median trends (SAS Proc. Quant. Reg.) showed statistically significant increases in bone marrow at day 15 ($p < 0.04$) and day 22 ($p < 0.01$), indicating a more rapid stem cell and marrow recovery in AED treated animals compared to vehicle controls. These results are evidence that AED could be successfully used to treat innate immune suppression by enhancing cell recovery in stem cell or bone marrow transplantation protocols. The $CD34^+$ stem cell data is shown below (AED is 'Neumune' in the graph below; TBI = total body irradiation).



17. Treatment of chemotherapy-induced innate immune suppression was examined using AED and another compound, 5-androstene-3β,7β,17β-triol (AET) in cynomolgous monkeys (*Macaca fascicularis*). Vehicle control AED and AET groups were treated beginning at 48 hours after a single administration of 35 mg/kg of carboplatin [diamine(1,1-cyclobutanedicarboxylato)platinum(II)] to induce innate immune suppression. The details of this protocol are described at Attachment 5. Each group consisted of three animals and this pilot study was therefore not powered to see small differences between the vehicle control group and the treated groups. Because of this, the results are expressed as qualitative differences. Animals treated with AED (60 mg/kg once per day for 10 days) never became neutropenic, while AET treatment (60 mg/kg once per day for 10 days) had a smaller qualitative effect and did not prevent a neutrophil drop below baseline levels compared to vehicle control animals.





18. Based on the collective data that is available to date, it is clear that AED can ameliorate innate immune suppression and increase neutrophils in non-human primates. Amelioration was observed as a decrease in the duration of

neutropenia, the severity of neutropenia or complete prevention of neutropenia. In healthy human adults, AED can significantly increase the numbers of neutrophils and platelets. To obtain these biological effects it is necessary to administer a sufficient amount of AED, either in a series of daily doses or in a single larger dose. The clinical data we obtained in adults showed that it was possible to see a neutrophil response by administering about 200 mg/day or 400 mg/day of AED in the 5 day dosing protocol discussed at paragraph 7. A 200 mg dose is 4 mg/kg for a person weighing 50 kg and 2 mg/kg for a person weighing 100 kg. It is clear that a single 30 mg AED dose as described in the '042 patent would not be effective in generating statistically significant increases in neutrophils in adult humans. In protocols where non-human primates were treated with a single dose of AED, it was necessary to administer a single large dose of at least about 40 mg/kg to see statistically significant amelioration of neutropenia. For a rhesus monkey weighing 4 kg, a 40 mg/kg dose amounts to 160 mg, which is well above the 30 mg maximum dose the '042 patent expressly discloses for AED. The results observed for AED activity were also unexpected in showing activity in a phase 1 clinical trial, where pharmacodynamic responses (neutrophil and platelet increases in this case) for drug candidates are often not seen in cases where pharmacodynamic responses are analyzed.

19. I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such false statements may jeopardize the validity of the application or any patent issued thereon.

Date: February 21, 2007 By: / Christopher L. Reading /

Christopher L. Reading

Attachment 1

Christopher Lewis Reading, Ph.D.
Curriculum Vitae (summary)

Vice President of Scientific Development
Hollis-Eden Pharmaceuticals
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Professional credentials and experience

- Ph.D. in Biochemistry, U.C. Berkeley, 1977
- Postdoctoral Fellowship in Tumor Biology, U.C. Irvine, 1978-1980
- Faculty, M.D. Anderson Cancer Center, 1980-1993
  Tenured Associate Professor of Medicine, Department of Hematology
  Stem Cell Transplantation and Gene Therapy
  Four granted patents in bispecific monoclonal antibodies and devices
- SyStemix, Inc. 1993-1998
- Vice President for Product and Process Development 1996-1998
  Senior Management team involved in sale of SyStemix, Inc. to Novartis
  Senior Director of Cellular Purification 1993-1996
  IND for autologous stem cell isolation for cancer
  IND for in utero transplantation
  IND for stem cell gene therapy for HIV
- Novartis Biotechnology Development and Production 1997-1998
- Cell and Gene Therapy Strategy
  Immune Cell Therapy Strategy
  Technical Analyst for Mergers and Acquisitions in Cell and Gene Therapy
  Technical Analyst for Intellectual Property in Cell and Gene Therapy
  Technical Analyst for Business Development and Licensing
  REV123 HIV Gene Therapy International Project Team
  GTI/SyStemix Technical Research and Development Integration Team
- Hollis-Eden Pharmaceuticals 1998-Present
- Vice President for Scientific Development
  IND for 16α-bromoepiandrosterone treatment of HIV
  IND for 3β, 7β, 17β-androstenetriol in vaccination of the elderly
  International clinical trial development for HIV, Malaria, HBV, HCV
  Established collaborations in South Africa, Thailand, Singapore and Australia
  Frequent presentations to Investment Bankers and Wall Street
- International Scientific Reputation 1977-2000
- 30 National and International Scientific Presentations
  77 publications in peer-reviewed journals

18 invited journal articles
20 invited book chapters
National Science Foundation Advisory Committee for SBIR Grants
Editorial Board, Journal Biological Response Modifiers
Editorial Board, Molecular Biotherapy
Peer-reviewed Grants and Contracts totaling over $2 Million
Consultant to Government Agencies and Private Corporations

Exemplary Published Articles in Peer-Reviewed Journals

Etkin, M., Filaccio, M., Ellerson, D., Suh, S. P., Claxton, D., Gaozza, E., Brenner, M., Moen, R., Belmont, J., Moore, K. A., Moseley, A. M., and Reading, C. (1992) Use of cell-free retroviral vector preparations for transduction of cells from the marrow of chronic phase and blast crisis chronic myelogenous leukemia patients and from normal individuals *Hum Gene Ther* **3**(2), 137-45

Moore, K. A., Deisseroth, A. B., Reading, C. L., Williams, D. E., and Belmont, J. W. (1992) Stromal support enhances cell-free retroviral vector transduction of human bone marrow long-term culture-initiating cells *Blood* **79**(6), 1393-9

Przepiorka, D., Giralt, S., Huh, Y. O., Andreeff, M., Luna, M., Reading, C., Thomas, M., and Champlin, R. E. (1992) T-cell-depleted autologous marrow fails to prevent acute graft-versus- host disease after allogeneic marrow transplantation for lymphoblastic lymphoma *Transplant Proc* **24**(6), 2906-7

Terstappen, L. W., Buescher, S., Nguyen, M., and Reading, C. (1992) Differentiation and maturation of growth factor expanded human hematopoietic progenitors assessed by multidimensional flow cytometry *Leukemia* **6**(10), 1001-10

Yuan, T., Zhou, Y. Q., Herst, C. V., Reading, C., Ellersen, D., Etkin, M., Khouri, I., Kantarjian, H., Talpaz, M., and Deisseroth, A. (1992) Molecular approaches to purging of chronic myelogenous leukemia marrow in autologous transplantation *Prog Clin Biol Res* **377**, 227-30

Zhang, W., Reading, C., and Deisseroth, A. B. (1992) Improved PCR sequencing with formamide *Trends Genet* **8**(10), 332

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Attachment 2

Human phase I single AED dose clinical protocol. The primary objectives of this study were: (1) to evaluate, relative to placebo, the safety and tolerance of four treatment groups (50 mg, 100 mg, 200 mg, and 400 mg) of androst-5-ene-3β,17β-diol (AED) or placebo equivalent when administered by intramuscular injection in healthy adult volunteers, and (2) to compare the pharmacokinetic profiles of AED at these dose levels after a single dose of the drug or vehicle placebo equivalent when administered by intramuscular injection in healthy adult volunteers. Thirty-two subjects were randomly assigned to one of four treatment groups (50 mg, 100 mg, 200 mg and 400 mg) of drug or placebo equivalent. Subjects were randomized within each treatment group to receive either active drug or an equal volume of placebo equivalent in a ratio of 3:1. Thus, for every three subjects who received the drug in a dose group, one subject received an equal volume of placebo equivalent. Eight subjects were selected for each treatment group (6 subjects to receive study drug and 2 to receive placebo). Within each treatment group there were to be 4 males and 4 females. The subjects in the first cohort (50 mg) were dosed first. Subjects were monitored for safety for 14 days post study drug administration. At the completion of Day 14, when safety had been reviewed and found to be acceptable, the second cohort (100 mg) was dosed. All subsequent dose groups proceeded in this manner.

After informed consent was obtained, subjects were screened for eligibility into the study. Subjects who met all entry criteria were asked to return on the day before dosing (baseline visit) to confirm ongoing eligibility and to check into the study Phase I unit. Study eligibility was established by medical history, prior medication history, safety laboratory evaluations, urine drug screen, and pregnancy testing. Safety laboratory evaluations included evaluations of chemistry, hematology, and urinalysis. Additionally, ongoing safety was assessed by physical examinations; vital signs collection, adverse event and injections site reaction collection, 12-lead ECG evaluation and concomitant medication collection. Subjects remained in the Phase I unit until all procedures were completed on the morning/afternoon of Study Day 6. Drug or placebo equivalent was administered once on Study Day 1. During the study, subjects underwent safety evaluations and provided blood and urine for safety laboratory testing. All subjects had blood samples collected at specified times on Study Days 1 through 6, 8, 14, 21 and 28 for pharmacokinetic analyses. Adverse events were evaluated daily and injection site reactions were evaluated throughout the study. During the follow-up, subjects were required to return to the study center on Study Days 8, 14, 21 and 28. The criteria for inclusion in the study included male or female subjects between 18 and 65 years of age (inclusive) who did not have any clinically significant medical abnormalities, chronic diseases, uncontrolled hypertension, malignancy, clinically-active serious infections or conditions, recent significant blood loss within 6 months of dosing, a clinically significant abnormal ECG, or an acute illness within 10 days prior to admission into the study.

AED was provided as a suspension formulation in vials. Each 1 mL of formulation contained 100 mg of drug. The vehicle consisted of mannitol,

polysorbate 80, benzalkonium chloride, sodium phosphate (monobasic and dibasic) and water for injection. All injection site irritation events were either mild or moderate in severity, and all were considered related to the drug. The most frequently reported adverse events considered unrelated to study medication were a hemoglobin decreased reported in 11 subjects; 34%, blood creatinine phosphokinase increased (10 subjects; 31%), blood sodium increased and white blood cells decreased (each reported by 5 subjects; 61%). There did not appear to be any dose-response relationship between the incidence of any of these events and the treatment group. The vast majority of events were mild (176 events, 93%) or moderate (12 events, 6%) in severity. For decreased white cell counts, three events occurred in the 8 placebo subjects, with 1 decrease considered treatment related , while the other two were not considered to be treatment related. A total of 4 decreased white cell count events were observed in the 24 subjects in the four dose level groups, with one being in the 400 mg dose group considered to be treatment related, while the other three events were not considered to be treatment related.

Human phase I multiple AED dose clinical protocol. This protocol was a Phase I, randomized, double-blind, placebo-controlled multiple, ascending dose study of AED administered to four different treatment groups by intramuscular injection in healthy adult subjects. Forty subjects were randomly assigned to one of four treatment groups (50 mg, 100 mg, 200 mg, and 400 mg groups) of drug or placebo equivalent. Subjects were randomized within each treatment group to receive either active drug or placebo equivalent in a ratio of 3:1 as in the study described above. Eight subjects were in each treatment group (6 subjects to receive study drug and 2 to receive placebo). An additional 8 subjects were enrolled into the 200 mg group after the last subject in the 400 mg group (2 x 2 mL) completed Day 56. Treatment groups were stratified by gender to assure approximately equal numbers of males and females. After informed consent was obtained, subjects were screened for eligibility into the study. Subjects who met all entry criteria were asked to return on the day before dosing (baseline visit) to confirm ongoing eligibility and to check into the study Phase I unit. Study eligibility was established by medical history, prior medication history, safety laboratory evaluations, urine drug screen, alcohol breath test, and pregnancy testing (females). Safety laboratory evaluations included evaluations of chemistry, hematology, and urinalysis. The subjects in the first cohort (50 mg) were dosed first. Subjects were monitored for safety for 14 days post study drug administration. At the completion of Day 14, after safety had been reviewed and found acceptable, the second cohort (100 mg) was dosed. All subsequent dose groups proceeded in this manner. Additionally, ongoing safety was assessed by physical examinations; vital signs collection, adverse event and injections site reaction collection, 12-lead ECG evaluation and concomitant medication collection. Subjects remained in the Phase I unit until all procedures were completed on Study Day 6. The drug was provided as a suspension, with 5 mL aliquots in 10 mL vials. Each 1 mL of formulation contained 100 mg of drug. The formulation vehicle consisted of mannitol, polysorbate 80, benzalkonium chloride,

sodium phosphate (monobasic and dibasic) and water for injection. Doses of drug in the study groups are summarized below.

Study Days	Intramuscular Injection	Group 1 (1 X 0.5 mL)	Group 2 (1 X 1 mL)	Groups 3 and 5 (1 X 2 mL)	Group 4 (2 X 2 mL)
1	1 Dose	50 mg	100 mg	200 mg	400 mg
2	1 Dose	50 mg	100 mg	200 mg	400 mg
3	1 Dose	50 mg	100 mg	200 mg	400 mg
4	1 Dose	50 mg	100 mg	200 mg	400 mg
5	1 Dose	50 mg	100 mg	200 mg	400 mg
Total	5 Doses	250 mg	500 mg	1000 mg	2000 mg

Analysis of data from this multidose trial included assessment of absolute neutrophil count, platelet count, reticulocyte count and hemoglobin. Two endpoints were analyzed, absolute change from baseline and percent change from baseline. Results were analyzed for significance, trends and other salient features. Several statistical tests were performed to quantify detectable signals. The Student t-test and the nonparametric Wilcoxon Mann-Whitney test were systematically applied to examine pair-wise differences from controls. Dose response trends at each time point were formally tested for significance through a simple linear regression model and the nonparametric Jonckheere Terpstra test. To force closure of an informal procedure, coupled with several tests and two endpoints, sensitivity of results was examined using the collective evidence at hand.

Attachment 3

Sub-lethal irradiation protocols. The sub-lethal protocol consisted of exposure of non-human primates, typically rhesus monkeys (*Macaca mulatta*) to a significant, but rarely lethal dose of radiation. For most of these studies, groups of animals were exposed to a target radiation dose of 400 cGy or 440 cGy (range 380 cGy-500 cGy) of whole body radiation. The monkeys were exposed to a total body irradiation (TBI) of low energy γ-radiation from a ^{60}Co source (energy levels of 1.173 and 1.332 million volts [MV]) or 6 MV photons from a linear accelerator. In these protocols, the animals generally experienced cytopenia associated with innate immune suppression, mainly a radiation-induced drop in neutrophils and a variable drop in platelets. The radiation doses of were selected for evaluation in sublethal studies based on literature on radiation doses in rhesus monkeys that could cause very severe neutropenia, which is defined as an absolute neutrophil count (ANC) of < 500 to 100 neutrophils/μL. For animals that received a 400 cGy radiation exposure and were not otherwise treated, there was a low death rate of about 2% at 30 days after the radiation exposure ($LD_{2/30}$). In several of the studies, animals that had counts of less than 500 neutrophils/μL were treated with antibiotics to prevent bacterial infection. The antibiotic treatment did not affect the course of neutrophil decrease or recovery in the animals.

The animals were transported to a radiation facility on the day of radiation by a carrier in an environmentally controlled vehicle. The animals usually received a midplane dose of about 400 cGy or 440 cGy at a dose rate of approximately 60 cGy/minute through simultaneous parallel opposed ^{60}Co sources. In a fourth study, the animals were irradiated using 6 MV X-rays from a linear accelerator.

Lethal irradiation protocols. The lethal radiation protocol consisted of exposure of non-human primates to a higher radiation dose (600-637 cGy) that was lethal for about 25-50% of animals that were not otherwise treated. Treating immune suppression in non-human primates after lethal radiation exposure has usually included (1) treatment of animals for overt or suspected bacterial infections and (2) transfusion of blood or blood products (usually platelets) for animals that have low blood element counts, and in some cases, (3) administration of parenteral nutrition or fluids to help the animals maintain their food consumption or electrolyte balance. For both the lethal and sub-lethal non-human primate protocols described here, procedures and protocols involving the care and use of the animals were reviewed and approved by applicable institutional review groups such as Institutional Animal Care and Use Committees prior to conduct. During the studies, the care and use of animals were conducted in accordance with applicable laws and regulations. Euthanasia criteria for animals *in extremis* were approved and implemented in accordance with applicable laws and regulations.

Most of the studies were conducted using a 600 cGy radiation exposure and one study was conducted with exposure to 634 cGy. The 600 cGy radiation dose was found to be about an $LD_{33/30}$ for animals that were not otherwise

treated. This higher radiation dose was used to evaluate the effect of AED on survival and on recovery of neutrophils and other cells or blood elements that mediate innate and other immune responses.

The formulations that were used in the studies summarized above are shown in the table below. Vehicle control groups were treated with the formulation lacking AED. The AED was present in the formulations as a suspension of particles of the sizes shown below.

Formulation & description	Composition	Particle Size
Macroparticle suspension (macrosuspension; research formulation)	2% polysorbate 80 0.9% w/v sodium chloride 0.1% w/v CMC* 0.05% v/v phenol	D (0.90): 84 μm**
Microparticle suspension (microsuspension; research formulation)	2% polysorbate 80 0.9% w/v sodium chloride 0.1% w/v CMC 0.05% v/v phenol	D (0.90): 5.5 μm
Nanoparticle suspension (nanosuspension)	2.2% w/v glycerin 0.5% w/v Poloxamer 188 0.2% w/v methylparaben 0.2% deoxycholic acid 0.14% sodium phosphate, dibasic	Mean (average): 0.6 μm 99% < 1.2 μm
Microparticle suspension	0.5% CMC 0.5% polysorbate 80 0.02% benzalkonium chloride 56.7 mM sodium phosphate, monobasic 5.9 mM sodium phosphate, dibasic pH 6.0	D (0.90): 13 μm
Microparticle suspension	4.8% mannitol 0.5% polysorbate 80 0.02% benzalkonium chloride 0.012% sodium phosphate, dibasic 0.08% sodium phosphate, monobasic pH 6.0 qs. with water for injection	D (0.90): 13 μm

* CMC = carboxymethylcellulose

** particle size before formulation in excipients; final formulation particle size not measured

Definitions of degrees of cytopenias are shown below.

Toxicity criteria for non-human primate hematologic conditions

Blood parameter	Grade					
	1	2	3	4	4+	5
neutrophils	LLN – 1,500/μL	<1,500 – 1,000/μL	<1,000-500/μL	<500 – 100/μL	<100/μL	Death
platelets	LLN – 75,000/μL	<75,000/μL – 50,000/μL	<50,000/μL – 25,000/μL	<25,000/μL – 10,000/μL	<10,000/μL	Death
hemoglobin	LLN – 10 g/dL	<10.0 – 8.0 g/dL	<8.0 – 6/5 g/dL	<6.5 – 4.0 g/dL	<4.0 g/dL	Death
description	Mild	Moderate	Severe	Very severe	ALF	Death

LLN = Lower limit of the normal range
ALF = Acutely life-threatening

Attachment 4

600 cGy irradiation with clinical support - analysis of bone marrow and stem cell recovery. Under these conditions, clinical support alters a radiation dose that would be expected to be about an $LD_{50/30}$ to a lower level of lethality. The eight animals (rhesus monkeys) used in this study weighed 2.5-4.0 kilograms and were at 2 to 3 years of age. The animals were irradiated with a single dose of 600 cGy from a 6 MV linear accelerator. During irradiation the monkeys were anesthetized and placed in a perspex frame. Results from this study are summarized in F.S.F. Kaya-Aerts et al., *Blood,* 106: abstract 4203, 2005, newly cited. Criteria for the use of supportive or clinical care in this study were as follows. Two weeks before irradiation, the animals were placed in a laminar flow cabinet and the gastrointestinal tract was selectively decontaminated by giving orally one single dose of Piperazine and Yomesan, starting at day 11 before TBI, followed by orally administered Flagyl, Madicure, and Diloxanide for 7, 5 and 10 days respectively, and Ciprofloxacin, Nystatin and Polymyxin B. This regimen was supplemented with systemic antibiotics, in most cases Ticarcillin and Cefuroxim, when leukocyte counts dropped below 1×10^9/L. Administration of antibiotics was discontinued when leukocyte counts rose above 1×10^9/L. During decontamination, iron supplementation, Venofer, was administered intravenously. Dehydration and electrolyte disturbances were treated by appropriate fluid and electrolyte administration subcutaneously. The monkeys received irradiated (20 Gy irradiation) platelet transfusions whenever platelet counts reach values below 40×10^9/L, packed red cells whenever hematocrit was lower than 20% and, occasionally, whole blood transfusions in case of coincidence of both transfusion criteria. The criterion of transfusion of platelets at counts $< 40 \times 10^9$/L was chosen because monkeys develop petechiae and other hemorrhages at this level. Platelet transfusions were prepared from adult male donor monkeys stimulated with thrombopoietin for 4 consecutive days. Bone marrow was aspirated under neurolept anesthesia using Ketalar (Apharmo, Arnhem, the Netherlands) and Domitor (Pfizer, Capelle a/d Ijssel, The Netherlands). Small bone marrow aspirates for analytical purposes were taken from the shafts of the femurs or humeri using pediatric spinal needles and collected in bottles containing 2 mL HEPES buffered Hanks' balanced salt solution (HBBS) with 200 IU sodium heparin/mL (Leo Pharmaceutical Products, Weesp, the Netherlands). Low density cells were isolated using a Lymphoprep (density 1.077) (Fresenius, Oslo, Norway) separation. Colony assays were conducted as follows. Cells were plated in 35-mm dishes in 1 mL enriched Dulbecco's medium containing 0.8% methylcellulose, 5% FCS, and additives. For burst-forming units-erythroid (BFU-E), cultures are supplemented with hemin (2×10^{-4} mol/L), human recombinant erythropoietin (Epo; 4 U/mL; Behring, Germany) and Kit ligand (KL; 100 ng/mL; Immunex Seattle, WA). For granulocyte/macrophage colony-forming units (GM-CFU), cultures are supplemented with recombinant human GM-CSF (5 ng/mL; Behring), recombinant rhesus monkey IL-3 (30 ng/mL), produced in *B. licheniformis* and purified as described previously, and KL. Low density cells are plated at 5×10^4 cells per dish in duplicate. Colony counts are calculated per mL of bone marrow

aspirated using the recovery of cells over the Ficoll density gradient. Colony numbers represented the mean ± standard deviation of bone marrow samples of individual monkeys. Hematological examinations were conducted as follows. Complete blood cell counts were measured daily using a ABC-vet animal blood counter (Scil, ABX diagnostics, Montpellier, France). For reticulocyte measurements, 5 μL EDTA blood was diluted in 1 mL PBS/EDTA/azide and 1 mL of a thiazole orange dilution was added, using thiazole in a final concentration of 0.5 g/mL. Measurements were done using a FACScan (Becton Dickinson, Leiden, The Netherlands) and analyzed using the Reticount software. Measurements of surface antigens were conducted as follows. Once weekly, a FACScan analysis was done on peripheral blood (PB) and bone marrow (BM) samples on the following surface antigens: CD8 and CD4 (T-cells), CD20 (B-cells), CD11b (myelomonocytes), CD56 and CD16 (NK cells) and CD34 (immature stem cells). HLA-DR was also measured on bone marrow cells. Directly labeled monoclonal antibodies were used for CD8, CD4, CD11b, CD20, CD56 and CD16 (Becton Dickinson) respectively. For CD34 a monoclonal antibody (mAb) against human CD34 (mAb 566, obtained from T. Egeland, University of Oslo, Oslo, Norway) that has been fluoresceinated with Cy-5 (Amersham, Biosciences, UK) according to standard procedures. A phyocoerythrin (PE)-conjugated mAb against human HLA-DR that reacts with rhesus monkey RhLA-DR antigens (Becton Dickinson) was used to measure HLA-DR activated CD34+ cells. A 0.5 mL sample of whole blood or bone marrow was lysed in 10 mL lysing solution (8.26 g ammonium chloride/1.0 g potassium bicarbonate and 0.037 g EDTA per L) for 10 minutes at 4 °C. After lysing the cells were washed twice with HBBS containing 2% BSA and 0.05% (wt/vol) sodium azide. The cells were resuspended in 100 μL of the latter fluid containing 2% normal monkey serum to prevent non-specific binding of the monoclonal antibodies. Monoclonal antibodies were added in a volume of 5 μL and incubated for 30 minutes on ice. After two washes, the cells were measured on the flow cytometer in the presence of propidium iodide (Sigma Aldrich, Zwijndrecht, The Netherlands). Ungated list mode data were collected for 10,000 events and analyzed using CellQuest software (Becton Dickinson). Statistical analysis was based on mean, median and range of numeric variables calculated on an Excel spreadsheet. Standard deviations were calculated on the assumption of a normal distribution. The statistical significance of differences was calculated with the Mann-Whitney test, comparing two unpaired groups each time. Comparisons were made between the treatment group and the placebo controls, as well as with historical controls of either untreated/placebo animals or those treated with other hematopoietic growth factors.

Attachment 5

Treatment of chemotherapy induced innate immune suppression. The animals (8 male and 4 female cynomolgus monkeys) weighed about 2 to 4 kg and were 2 to 6 years of age. A 35 mg/kg carboplatin dose was administered to the animals as a 10 mg/mL in 0.9% sodium chloride by continuous infusion for approximately 30 minutes using a Harvard syringe infusion pump. The animals were treated subcutaneously at interscapular region of the back once per day for 10 consecutive days with an AED or androst-5-ene-3β,7β,17β-triol (AET) suspension formulation or vehicle control. The daily AED dose of 170 mg (~60 mg/kg) was a suspension in 0.1% w/v carboxymethyl-cellulose, 0.9% w/v sodium chloride, 0.05% v/v phenol. The daily AET dose of 170 mg (~60 mg/kg) was a suspension in 40% v/v PEG200, 2% v/v benzyl benzoate, 2% v/v benzyl alcohol, qs propylene glycol (~56%). The day before dosing was designated Day –1. The first day of dosing was designated Day 1. Blood was collected on Days –5, 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, 27, 29, 31 and 34 before dosing. Blood samples of about 1.3 mL were drawn using a butterfly infusion set or syringe and needle from the saphenous, femoral or cephalic vein. Blood was transferred to a tube treated with dipotassium EDTA and used for the measurement of blood parameters such as erythrocyte count, leukocyte count, hematocrit value, hemoglobin concentration, platelet count, reticulocyte count and differential leukocyte count (absolute and percentage). Cellularity and differential of bone marrow biopsy was examined on three occasions from each animal at Day –5, approximately Day 15 and Day 34. Bone marrow biopsies were collected after blood samples. Bone marrow cores, touch preparations and aspirates as needed were collected from each animal from the head of the humerus, femur or iliac crest. The approximately Day 15 biopsy was taken on the day of the next scheduled blood collection for hematology analysis following two consecutive increases in any group peripheral blood mean neutrophil count after the group mean nadir. Bone marrow smears (4 or more/sample) were prepared by placing a drop of aspirate on a slide, covering with a second slide, pulling apart and air drying before Wright's staining. Groups of three animals (two males and one female) per group were selected as follows: (1) animals that received the AET vehicle control once per day for 10 consecutive days, (2) animals that received AED once per day for 10 consecutive days and (4) animals that received AET once per day for 10 consecutive days.